| Bauer Core Standard Protocol | | |
|---|---|---|
| Title: Using the Covaris S220 to shear DNA in a micro tube | | |
| Pages: 2 | Revision: 2.0 | Date: 3/9/16 |
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**1. Purpose**

This protocol provides instructions for using the Covaris S220 shearing instrument to shear nucleic acid samples. The protocol is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

**2. Materials**

2.1. Sample(s) to be sheared
- 2.1.1. Sample quantity: up to 10 µg
- 2.1.2. Sample volume: 130µl or 50 µl

2.2. Microtube (6 x 16 mm) part # 520045

**3. Instrumentation** Covaris S220

**4. Reagent preparation** None.

**5. Procedure**

5.1. Start Up: Allow 30 minutes for start up procedure.
- 5.1.1. Turn on the chiller beneath the bench (power switch on back).
  - 5.1.1.1. Set to 2.5 $^{o}$C to give water at ~6$^{o}$C in the Covaris water bath.
  - 5.1.1.2. To adjust, push the knob, turn to desired temp, and push again.
- 5.1.2. Fill the Covaris water bath with DI $H_2O$.
- 5.1.3. Run the SonoLab software.
- 5.1.4. Allow the water bath to degas and come to temperature before running.

5.2. Creating a New Method
- 5.2.1. Refer to library prep protocol for settings, or select according to the tables below:

**For 130ul**

| Target BP | 150 | 200 | 300 | 400 | 500 | 800 | 1000 | 1500 |
|---|---|---|---|---|---|---|---|---|
| Duty Factor | 10% | 10% | 10% | 10% | 5% | 5% | 5% | 2% |
| Peak Incident Power | 175 | 175 | 140 | 140 | 105 | 105 | 105 | 140 |
| Cycles Per Burst | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Duration | 430 | 180 | 80 | 55 | 80 | 50 | 40 | 15 |

**For 50ul**

| Target BP | 150 | 200 | 300 | 400 | 500 | 1000 | 1500 |
|---|---|---|---|---|---|---|---|
| Duty Factor | 10% | 10% | 10% | 5% | 5% | 2% | 1% |
| Peak Incident Power | 175 | 175 | 175 | 175 | 175 | 175 | 175 |
| Cycles Per Burst | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Duration | 280 | 120 | 50 | 55 | 35 | 45 | 20 |

5.3. Preparing the Sample Tube

5.3.1. Load the sample though the septum into the tube with a pipette.

5.3.2. Tubes are designed for 130µl but can also accomodate 50 µl.

5.3.3. Check for air pockets and spin down if present.

5.3.4. Place the tube into the plunger holder so that it sits straight.

5.3.5. Load the plunger holder into the instrument (any direction).

5.4. Running a Saved Method.

5.4.1. Wait for the instrument status to be ready and then hit Run.

5.5. Cleanup

5.5.1. Remove the sample tube.

5.5.2. Turn off the degas pump

5.5.3. Empty the water tank.

5.5.4. Run the degas pump to purge residual water from the lines.
The pump will automatically stop after 10 seconds.

5.5.5. Empty residual water from the tank and allow to air dry.